

Supplemental Methods

Laboratory assessments

The performance of both the Roche and Centaur assays for estimating TSH show very good agreement with a previous analysis demonstrating a regression coefficient (95% confidence intervals) of 0.99 (0.99 – 0.99) (1). Similarly, both hs cardiac troponins are markers of myocardial damage and necrosis and have high correlation ($r=0.89$) in patients presenting with suspected AMI (2).

Groups of FT3 and CRP

Various combinations of tertiles of FT3 and CRP levels were evaluated and classified as follows:

Group A: Low FT3 (<4.4 pmol/L; lowest tertile) and high CRP (>6.0 mg/L; highest tertile)

Group B: Low FT3 (<4.4 pmol/L; lowest tertile) and low CRP (<1.0 mg/L; lowest tertile)

Group C: Moderate FT3 (4.5 to 5.0 pmol/L) and low, moderate and high CRP

Group D: High FT3 (>5.0 pmol/L; highest tertile) and high CRP (>6.0 mg/L, highest tertile)

Group E: High FT3 (>5.0 pmol/L; highest tertile) and low CRP (<1.0 mg/L; lowest tertile)

Missing data imputation

Multiple imputation by chained equations was used to impute 5 complete datasets and results were pooled. All variables used in the analysis models were included in the imputation. Models were run with and without imputation of missing data. The results obtained from both analyses were qualitatively similar and thus the pooled imputed data analysis was classed as the main analysis and the complete case results were analysed as a sensitivity analysis.

Mediation analysis

Mediation is the process through which an exposure causes disease. Researchers may hypothesize that some or all of the total effect of exposure (X) on an outcome (Y) operates through a mediator (M), which is an effect of the exposure and a cause of the outcome. When a mediator is hypothesized, the total effect can be broken into two parts: the direct and indirect effect. The direct effect is the effect of exposure on the outcome absent the mediator. The indirect pathway is the effect of exposure on the outcome that works through the mediator. Causal mediation analysis allows for effect decomposition in the presence of X-M interaction by defining direct and indirect effects (controlled or natural) from a potential outcomes framework and developing estimations of these quantities that are not model specific (**eFigure 1**). Causal mediation elucidates the four main assumptions for estimating direct and indirect effects. The causal mediation approach requires the conduction of sensitivity analyses to examine the robustness of findings to violations of these assumptions.

There four main assumptions in causal mediation analysis are:

1. There is no unmeasured $X \rightarrow Y$ confounding.
2. There is no unmeasured $M \rightarrow Y$ confounding.
3. No unmeasured $X \rightarrow M$ confounding.
4. No $M \rightarrow Y$ confounding caused by X.

The R causal mediation package (mediation) uses simulations to estimate direct and indirect effects when there is X-M interaction (3). It does so by modeling the interaction in the outcome regression model and using the `mediate ()` function to estimate the natural direct and indirect effects based on Pearl's mediation formula (4). If investigators are unsure about whether they should model X-M interaction, a formal test of X-M interaction may be conducted using the function `test.TMint ()`; a significant finding implies that the no X-M interaction assumption does not hold. To address the second limitation of the traditional approach, a sensitivity analysis function, `medsens ()`, allows for investigators to examine, through simulations, the

robustness of their findings to potential unmeasured M-Y confounders. Results for all analyses are displayed using the summary () and plot () functions.

Sensitivity analysis is performed to examine whether the results are robust to the violation of the assumption of sequential ignorability. Violation to assumption indicates that there exists an unmeasured confounder that is related to both the mediator and the outcome. Note that this unmeasured confounder is not affected by the treatment. Sensitivity analysis uses certain statistics to quantify how strong the confounder would have to be to change the conclusion being drawn about the direct and indirect effect (5). The correlation parameter (ρ) reflects the existence of omitted variables that were related to the mediator and outcome even after conditioning on treatment, and the parameter was added to the calculations of ACME. Sensitivity analysis is to vary ρ values and compute corresponding ACMEs.

Sensitivity analysis

To ensure the validity and robustness of the results obtained, we investigated the relationship between FT3 levels and all-cause mortality by performing the following sensitivity analyses: (1) separately for each of the two thyroid function assays utilised, (2) by excluding LT4 users, (3) by type of AMI (STEMI or NSTEMI), (4) by sex of participants, (5) by type of troponin (T or I) assay used, (6) by analysing complete case results only (available for 1883 participants) without imputation of missing data, (7) by assessing if FT3 may mediate the association between CRP and mortality, and (8) by analyzing the relationship between FT3, CRP and all-cause mortality in only the participants with blood samples obtained on admission and prior to coronary angiography.

References:

1. Kahapola-Arachchige KM, Hadlow N, Wardrop R, Lim EM, Walsh JP. Age-specific TSH reference ranges have minimal impact on the diagnosis of thyroid dysfunction. Clin Endocrinol (Oxf). 2012;77:773-9.

2. van der Linden N, Wildi K, Twerenbold R, Pickering JW, Than M, Cullen L, Greenslade J, Parsonage W, Nestelberger T, Boeddinghaus J, et al. Combining High-Sensitivity Cardiac Troponin I and Cardiac Troponin T in the Early Diagnosis of Acute Myocardial Infarction. *Circulation*. 2018;138:989-999.
3. Tingley D, Yamamoto T, Hirose K, Keele L, Imai K. Mediation: R package for causal mediation analysis. 2014 <http://cran.r-project.org/web/packages/mediation> Accessed 12th October 2019
4. Pearl J. Direct and indirect effects. Proceedings of the seventeenth conference on uncertainty in artificial intelligence, 2001. Morgan Kaufmann Publishers Inc., 411-420. (section 3)
5. VanderWeele TJ. Mediation Analysis: A Practitioner's Guide. *Annu Rev Public Health*. 2016;37:17-32.

Supplemental Results

Supplemental Table 1. Details of the relationship between various parameters assessed in the main regression model and all-cause mortality

Parameter	HR (95% CI)*	P value
Centre	1.05 (1.02 to 1.09)	0.006
Male sex	1.21 (1.07 to 1.36)	0.002
Age, years	1.09 (1.08 to 1.09)	<0.0001
Ethnicity		
White (reference)	1.0	0.003
South Asian	1.11 (0.45 to 2.71)	
Black	n/a	
Other†	5.41 (2.21 to 13.25)	
Body mass index, kg/m ²	1.00 (0.92 to 1.06)	0.71
Smoking status		
Never smokers (reference)	1.0	<0.0001
Ex-smokers	1.23 (0.95 to 1.43)	
Current smokers	1.78 (1.53 to 2.07)	
NSTEMI (reference)	1.0	0.001
STEMI	0.79 (0.68 to 0.91)	
Adjusted LVEF‡, %	1.01 (1.01 to 1.02)	<0.0001
Serum creatinine, micromole/L	1.13 (1.11 to 1.15)	<0.0001
z-troponin§, unit	1.11 (1.05 to 1.18)	<0.0001
CRP, mg/L	Nonlinear	<0.0001
FT3, pmol/L	Nonlinear	<0.0001
Pre-existing medical conditions		
Ischemic heart disease	1.08 (0.83 to 1.04)	0.20
Hypertension	1.02 (0.91 to 1.14)	0.68
Type 2 diabetes mellitus	1.77 (1.57 to 1.99)	<0.0001
Atrial fibrillation	1.65 (1.38 to 1.91)	<0.0001
Hypothyroidism on LT4 therapy	1.02 (0.85 to 1.22)	0.83

* Adjusted for all the variables listed in this table.

†Others comprised of people of Chinese, Middle Eastern and rest of ethnic groups not covered by the White South Asian and Black categories.

‡LVEF adjusted for time interval (in days) between date of acute myocardial infarction and echocardiography.

§ standardised, centred and combined troponin (T and I) values termed z-troponin.

|| the hazard ratios for FT3 and CRP had a non-linear relationship with all-cause mortality (details are provided in Figure 2 and online Figure 3)

CRP – C reactive protein; FT3 – free triiodothyronine; HR – hazard ratio, LT4 – levothyroxine; LVEF – left ventricular ejection fraction NSTEMI – non ST elevation myocardial infarction; STEMI – ST elevation myocardial infarction.

Supplemental Table 2. Association of CRP with serum TSH or FT4 levels

	Unstandardized Coefficients	95% confidence interval	P value
TSH mIU/L	-0.28	-0.62 to 0.06	0.11
FT4 pmol/L	0.27	-0.03 to 0.58	0.08
FT3/FT4	-0.01	-0.02 to 0.009	0.04

Adjusted for age, sex, centre, ethnicity, body mass index, smoking status, type of acute myocardial infarction (STEMI or NSTEMI), adjusted LVEF^{*}, ischemic heart disease, type 2 diabetes mellitus, hypertension, atrial fibrillation, hypothyroidism, serum creatinine, and z-troponin.

^{*}adjusted for interval between date of acute myocardial infarction and date of echocardiogram.

CRP – C reactive protein, FT4 – free thyroxine, FT3/FT4 – free triiodothyronine to free thyroxine ratio, LVEF – left ventricular ejection fraction, NSTEMI – non ST elevation myocardial infarction, STEMI – ST elevation myocardial infarction, TSH – thyrotropin, z-troponin - standardised, centred and combined troponin (T and I) values.

Supplemental Table 3. Relationship between serum TSH or FT4 with all-cause mortality

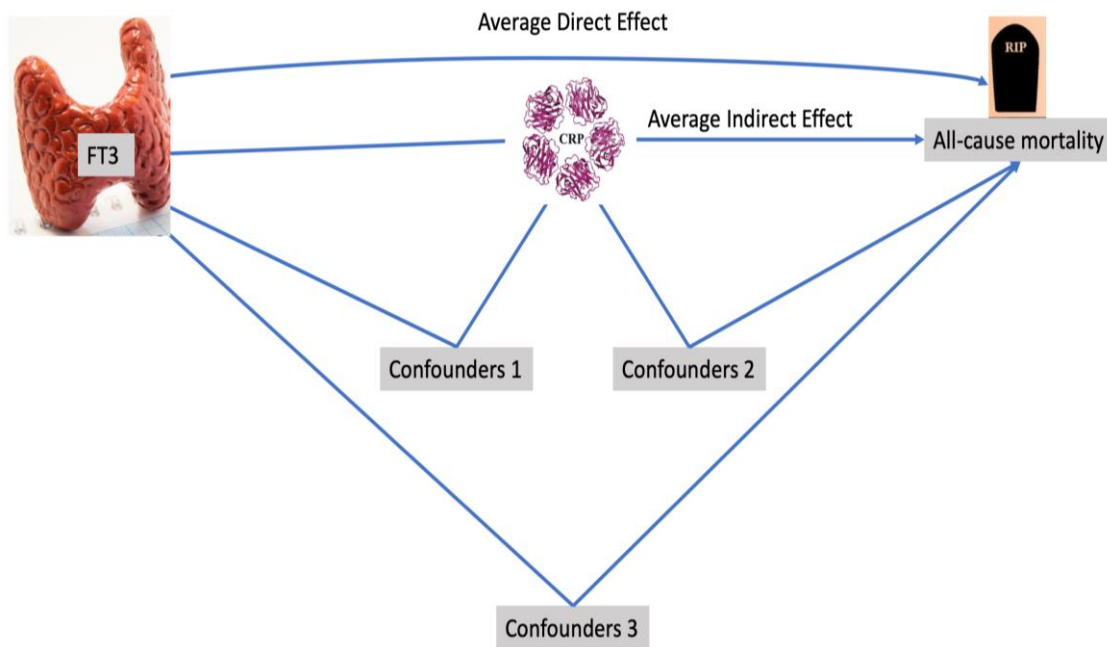
	HR (95% CI)	P value
TSH mIU/L	0.99 (0.96 to 1.03)	0.91
FT4 pmol/L	1.04 (1.01 to 1.07)	0.008
FT3/FT4	0.64 (0.50 to 0.80)	0.0002

Adjusted for age, sex, centre, ethnicity, body mass index, smoking status, type of acute myocardial infarction (STEMI or NSTEMI), adjusted LVEF^{*}, ischemic heart disease, type 2 diabetes mellitus, hypertension, atrial fibrillation, hypothyroidism, serum creatinine, CRP, and z-troponin.

^{*}adjusted for interval between date of acute myocardial infarction and date of echocardiogram.

CRP – C reactive protein, FT4 – free thyroxine, FT3/FT4 – free triiodothyronine to free thyroxine ratio, LVEF – left ventricular ejection fraction, NSTEMI – non ST elevation myocardial infarction, STEMI – ST elevation myocardial infarction, TSH – thyrotropin, z-troponin - standardised, centred and combined troponin (T and I) values.

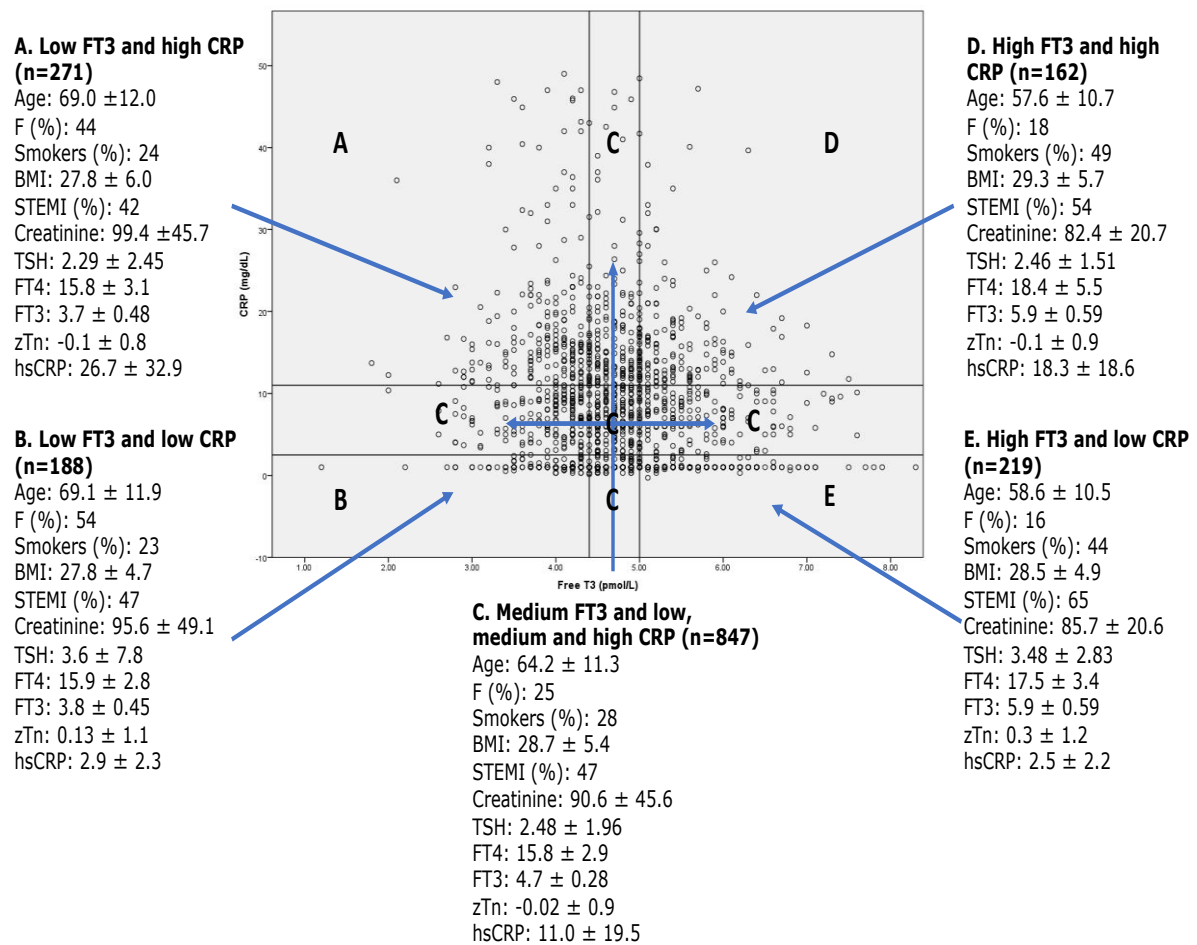
Supplemental Figure 1. Directed acyclic graph symbolically representing the relationship between FT3, hsCRP and all-cause mortality in the ThyAMI-1 cohort



Path diagram for a survival mediation model in the two-stage framework with exposure FT3, mediator CRP, outcome all cause mortality and confounders (covariates).

Supplemental Figure 2. Baseline characteristics of patients from the ThyAMI-1 study

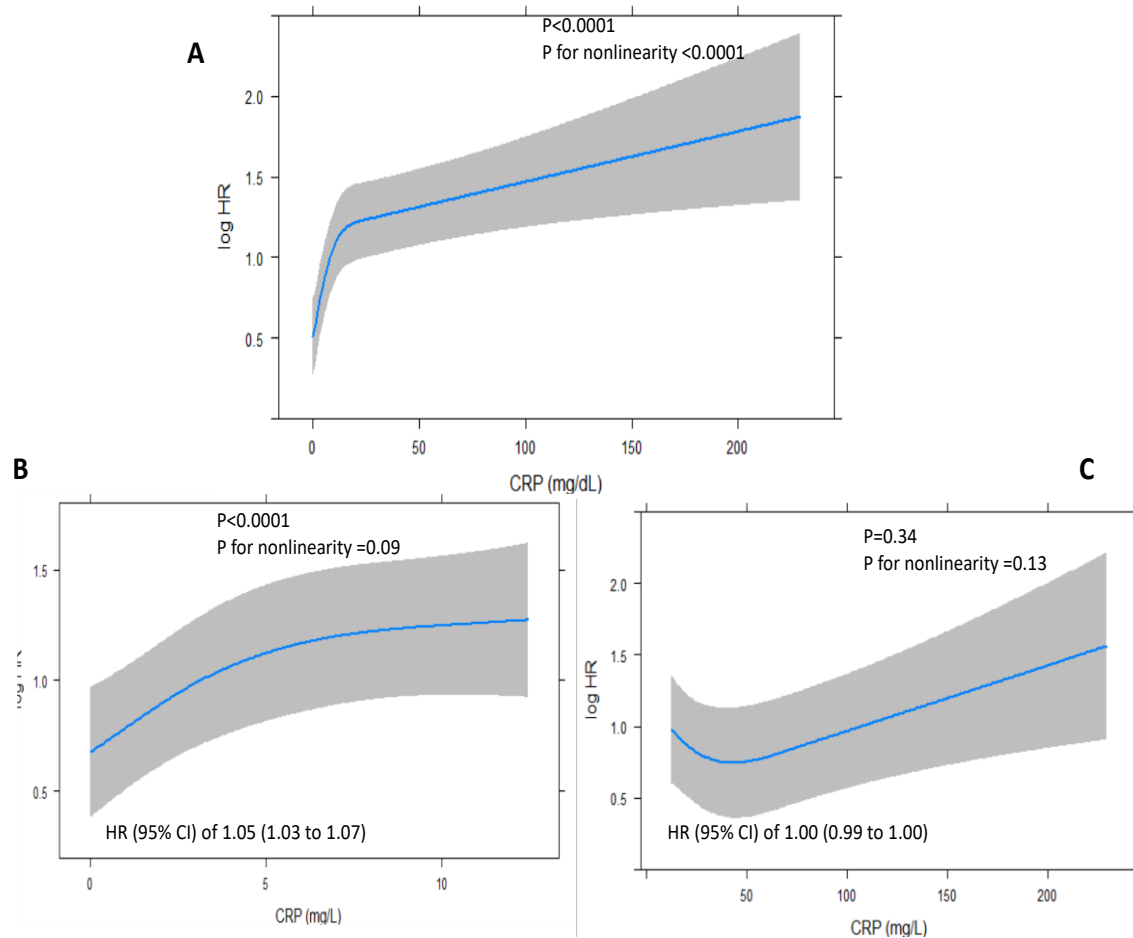
(n=1687*) in relation to FT3 and CRP levels



*Complete case data available only. Presented as mean (SD) or frequencies (%).

F – females; BMI – body mass index; STEMI – ST elevation myocardial infarction; TSH – thyrotropin; FT4 – free thyroxine; FT3 – free triiodothyronine; zTn – standardised and combined troponin values (T and I); hsCRP – high sensitivity C reactive protein.

Supplemental Figure 3. Relationship between baseline CRP levels in all participants with acute myocardial infarction (A) and by baseline CRP levels of ≤ 12.5 mg/L (B) or >12.5 mg/L (C) with all-cause mortality

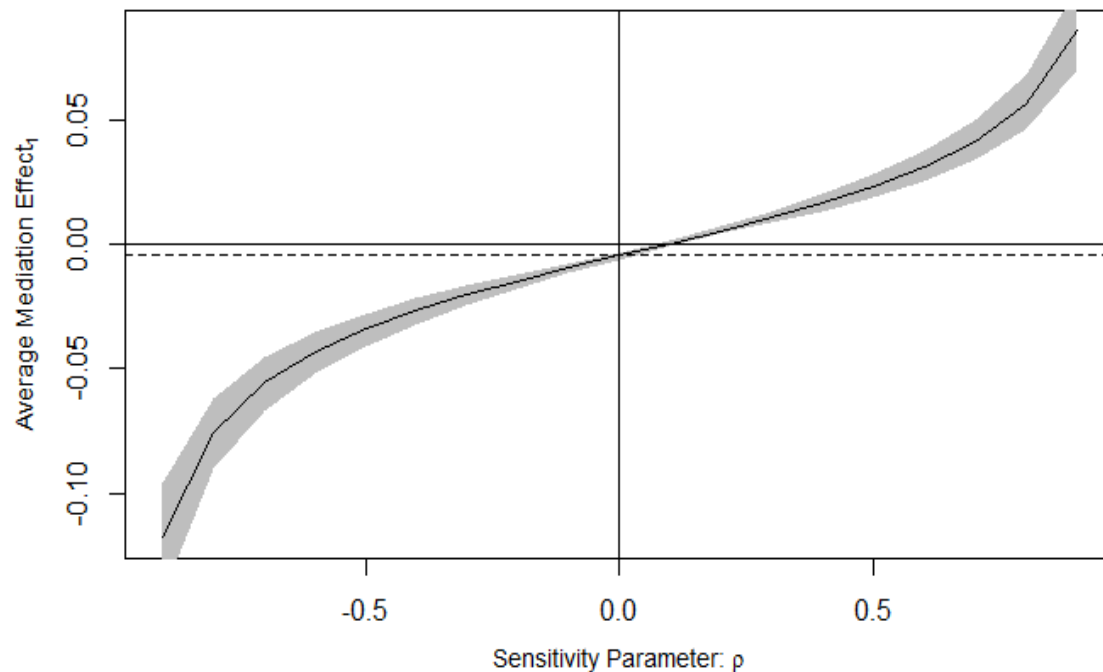


Adjusted for age, sex, centre, ethnicity, body mass index, smoking status, type of acute myocardial infarction (STEMI or NSTEMI), adjusted LVEF*, ischemic heart disease, type 2 diabetes mellitus, hypertension, atrial fibrillation, hypothyroidism, serum creatinine, free T3, and z-troponin.

CRP – C reactive protein; NSTEMI – non ST elevation myocardial infarction; STEMI – ST elevation myocardial infarction; LVEF – left ventricular ejection fraction; T3 – triiodothyronine; z-troponin – standardised and combined troponin values (T and I).

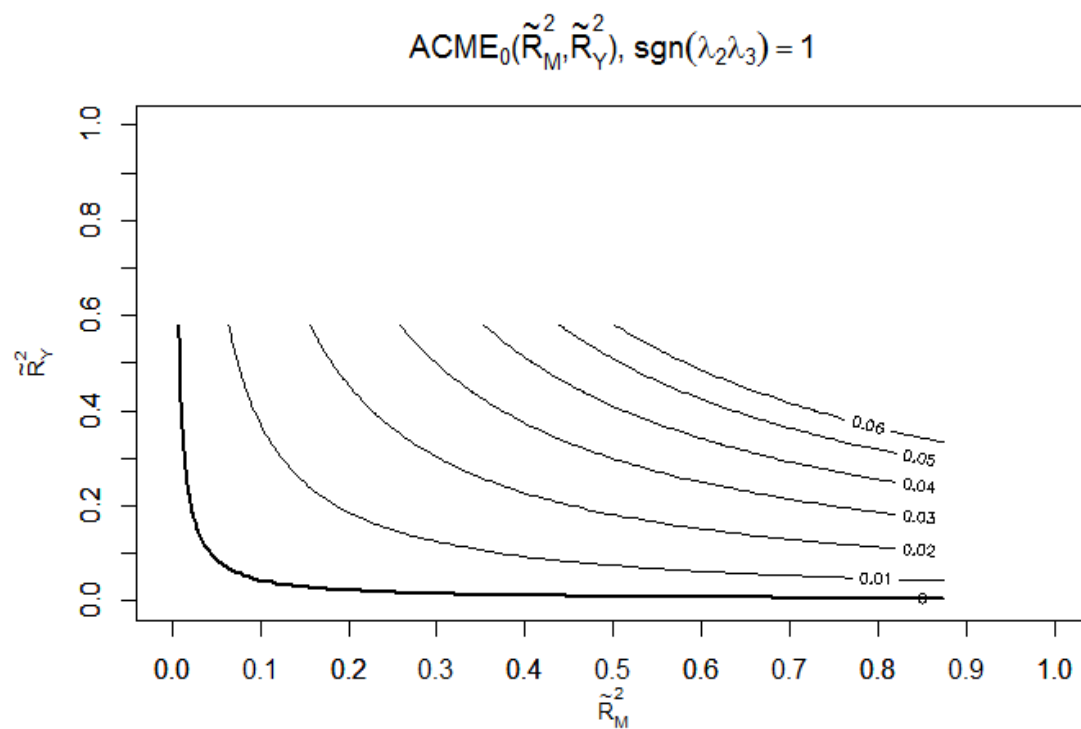
*adjusted for interval between date of acute myocardial infarction and date of echocardiography

Supplemental Figure 4. Sensitivity analysis lot of the average causal mediation effect as a function of the correlation parameter ρ



Sensitivity analysis plot varies the average causal mediation effect (ACME) values with the corresponding sensitivity parameter (ρ). The ρ reflects the existence of omitted variables that were related to the mediator and outcome and the parameter was added to the calculations of ACME. The solid line represents the estimated mediation effects, and the grey areas represent the 95% CIs from the bootstrap method at each value of the sensitivity parameter ρ . The dashed horizontal line represents the estimated mediation effect under the sequential ignorability assumption displaying the values of ρ at which the confidence intervals contain zero for the ACME. As can be observed, at no value of ρ is zero included within the confidence interval (grey area).

Supplemental Figure 5. Sensitivity analysis plot of the mean square error



Sensitivity analysis plots as a function of the standard deviation of the mean square error R^2 . \tilde{R}_M^2 is the square of the correlation between independent variables ($M \rightarrow X$), while \tilde{R}_Y^2 is the square of the correlation between dependent and independent variables ($Y \rightarrow X, M$).